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<p>This report summarizes progress for the first year of grant AFOSR-88-0033-07 entitled "Adaptation and Resistance of Ecosystems to Stress." This research employs naturally-derived, microbial microcosms to evaluate the ability of aquatic communities to adapt to stress. A series of laboratory toxicity tests have been conducted examining structural and functional responses of microbial communities to a sequence of stresses. Tentative conclusions from early research suggest that there is no detectable acclimation at the community level after low level cadmium exposure. More severe stress appears to be necessary to produce detectable increases in tolerance to subsequent stress. Acclimation was observed for net daily metabolism of communities developed in zinc and subsequently exposed to zinc, for gross primary production and respiration in communities colonized in zinc and then exposed to cadmium; and for species richness of communities colonized in zinc and then exposed to acidic pH. (AW)</p>								
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Adaptation and Resistance of Ecosystems to Stress

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to the  
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# SUMMARY

This report summarizes progress for the first year of grant AROBR-88-0033-07 entitled "Adaptation and Resistance of Ecosystems to Stress". This research employs naturally-derived, microbial microcosms to evaluate the ability of aquatic communities to adapt to stress. A series of laboratory toxicity tests have been conducted examining structural and functional responses of microbial communities to a sequence of stresses. Tentative conclusions from early research suggest that there is no detectable acclimation at the community level after low level cadmium exposure. More severe stress appears to be necessary to produce detectable increases in tolerance to subsequent stress. Acclimation was observed for net daily metabolism of communities developed in zinc and subsequently exposed to zinc, for gross primary production and respiration in communities colonized in zinc and then exposed to cadmium, and for species richness of communities colonized in zinc and then exposed to acidic pH.

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## INTRODUCTION

This report summarizes progress for the first year of grant AFOSR-88-0033-07 entitled "Adaptation and Resistance of Ecosystems to Stress". The purpose of this research is to evaluate the ability of communities to adapt to stress. Adaptation and decreased resistance have been observed at the population level (e.g. Cairns et al. 1976, Duncan and Klaverkamp 1983, Weis and Weis 1989). But, although there is much speculation about unique mechanisms of adaptation possible at levels of biological organization higher than the population, there have been few experiments examining the adaptation of communities to stress (e.g., Fisher 1977, Blanck and Wangberg 1988). Because multiple insults to natural systems are quite common, adaptation and resistance are likely to be important factors in determining the response of natural systems to anthropogenic stress. An understanding of the abilities of ecosystems to adapt to stress, or of possible multiplicative effects of repeated insult, may improve the ability to predict the effects of human activities on natural systems.

This research employs microbial communities developed on artificial substrates as microcosms to evaluate the ability of aquatic communities to adapt to stress. These communities are naturally-derived and can be easily be maintained in the laboratory, providing a practical experimental unit for studies on the response of communities to repeated stress.

## OBJECTIVES

The original objectives of this study were to:

1. determine whether communities with a history of stress respond differently to subsequent stress than do unstressed communities,
2. determine whether adaptations are generalizable from one type of stress to another,

We also identified two possible sub-objectives which are contingent on observations of adaptation in laboratory tests:

3. characterize possible mechanisms of observed adaptations,
4. and, analyse commonalities between stresses to which communities adapt.

## RESEARCH PROGRESS

The first year of research has involved range-finding tests for a variety of stresses, and the screening of combinations of colonization conditions and subsequent stresses to identify adaptive responses. Two groups of tests have been completed. First, full scale comparative toxicity tests have been conducted comparing the acute and chronic sensitivity to zinc of control communities and communities developed under a minimal cadmium stress. Second, screening tests have compared the response of communities developed under 3 levels of zinc stress to subsequent acute exposure to a variety of chemicals at a level sufficient to reduce taxonomic richness by approximately 50%.

## Materials and Methods

Periphytic communities were obtained by suspending polyurethane foam (PF) artificial substrates (6.0 x 5.0 x 3.75 cm) just below the surface in a 1 m depth in Pandapas Pond, a man-made impoundment in Jefferson National Forest, Montgomery County, VA. The substrates colonized for >14 d, then were retrieved, transported to the laboratory, and acclimated to laboratory temperatures at the rate of 2°C per day. One naturally-derived substrate was suspended in the center of each test chamber of high density polyethylene (35 x 28 x 15 cm) containing 7.5 L of test media. These naturally-derived substrates served as epicenters (i.e., species exporting units) and provided a source of colonists for the development of new communities on six initially barren, island substrates suspended around the epicenter in colonization tanks (Figure 1).

Colonization and test media used carbon dechlorinated tap water as diluent (mean hardness 70 mg/L, pH 7.7). Stressed communities were developed in water amended with a stock solution of cadmium chloride or zinc sulfate. Media was renewed 1 x weekly in early tests. In later tests media was replaced continuously by peristaltic pump with volume replacement in 2.4 d. Light was provided by 4 4' Vita-lite full spectrum bulbs (color rendering index > 90, Durotest Corp.) yielding a photon flux density (400-700 nm) at the water surface of 100  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Both colonization and toxicity tests were conducted at ambient room temperatures that ranged from 18.0 to 23.0°C. Photoperiod was 12 h light and 12 h dark. Island communities were colonized for 3 wk before being exposed to a secondary stress.

In the full scale acute test, substrates were colonized under two conditions. Control substrates were colonized in unamended dechlorinated tap water. Stressed substrates were developed in water amended with  $1 \text{ ug Cd L}^{-1}$ . This level of cadmium reduced protozoan species richness by 20% in previous tests (i.e., the inhibitory concentration for 20% or IC20), and approximated the lowest observable effect concentration (LOEC) when compared to the control, for a number of responses (Niederlehner et al. 1985, Table 1). Substrates from the two pretreatment groups were randomly assigned to 6 posttreatment groups: exposures of 0, 500, 1,000, 2,000, 4,000, 8,000, and 16,000  $\text{ug Zn L}^{-1}$ . Each substrate was placed in a 600 ml borosilicate glass beaker containing 500 ml of media. Substrates were exposed for 48 h. Each treatment was replicated four times. Replicates were set up over a 10 d period with replicates of each treatment interspersed through time according to a partially confounded split plot design.

In the full scale chronic test, substrates from each of the two pretreatment groups, i.e., control and  $1 \text{ ug L}^{-1}$  cadmium stressed, were randomly assigned to 6 posttreatment groups consisting of zinc exposures of 0, 10, 32, 100, 320, 1,000, and 3,200,  $\text{ug Zn L}^{-1}$ . Each substrate was suspended at one end of a 6 L glass aquarium (25 x 16 x 16 cm) containing 5 L of media. One initially barren PF substrate was suspended at the other end. After a 7-d exposure, the island substrates were sampled. Each treatment was replicated three times. Replicates were blocked



over time so that one complete set of treatments was set up every other day over 5 days.

In a series of screening tests, substrates were developed under three stress conditions. The unstressed pretreatment had no added zinc. A low stress group was dosed with  $50 \text{ ug Zn L}^{-1}$ , the IC30 for protozoan species richness in chronic exposure, and a moderate stress group was dosed with  $225 \text{ ug Zn L}^{-1}$ , the chronic IC50 based on previous tests (Pratt et al. 1987b, Table 1). Substrates from each pretreatment group were randomly assigned to two posttreatment groups, a control and the acute IC50 of toxicant based on preliminary range-finding tests (Table 2). Replication was blocked over time and one complete set of treatments was set up each week for three sequential weeks. Each substrate was placed in a 600 ml borosilicate glass beaker containing 500 ml of media. Substrates were exposed for 48 h.

After exposure to the secondary stress, several responses of communities were monitored. The substrate was removed from the test tank and destructively sampled. Material from the interstices of the substrate was collected by decanting excess liquid, then squeezing each substrate over a sterile 250-ml polystyrene sample cup, resubmerging the substrate, and squeezing again to dryness.

Species richness of protozoan communities was determined within 36 h of sampling by microscopic examination of live samples. Two to three drops of material from the bottom of the beaker were pipetted onto microscope slides and covered with a No. 1, 22-mm coverglass. The entire coverglass was scanned at 200 and/or 450X total magnification. Protozoans were

distinguished to the lowest practical taxon based on gross morphology and behavior (Curds 1982, Curds et al. 1983, Huber-Pestalozzi 1941-1968, Kahl 1930-1935, Lee et al. 1985, Page 1976, Pascher 1913-1927). Generally, identification to genus or species was possible. If taxonomic identification was uncertain, observations and drawings or photomicrographs were made to ensure consistent identification over the course of the experiment. Subsamples from each sample were examined until an asymptotic number of species was reached. Generally, two subsamples were required and >1000 individuals were identified.

Nontaxonomic responses of substrate communities to stress were monitored. In vivo fluorescence (IVF) was measured on a Turner Designs fluorometer, expressed as fluorescent units (FU), and used as an index of algal biomass (Lorenzen 1966). Ash free dry weight (AFDW) was determined by filtering a 10 ml aliquot on a preashed GF/A glass fiber filter, drying at 105 ° C for 24 h, weighing to the nearest 0.01 mg on a Cahn electrobalance, ashing at 500 ° C for 1 h, wetting the filters, redrying, and reweighing them. AFDW was expressed as mg AFDW L<sup>-1</sup> of substrate contents and used as an index of total biomass. Respiration to biomass ratios were monitored using the dehydrogenase assay developed by Owens and King (1975) and expressed as ug O<sub>2</sub> mg AFDW<sup>-1</sup> h<sup>-1</sup>. In screening tests gross primary production (GPP as mg O<sub>2</sub> beaker<sup>-1</sup> 12 h<sup>-1</sup>) and community respiration (CR as mg O<sub>2</sub> beaker<sup>-1</sup> 24 h<sup>-1</sup>) were estimated from dissolved oxygen measurements during the later part of the exposure. After 24 h, the beaker was covered with gas impermeable plastic film (e.g., Saran Wrap; Giddings and

Eddelmon 1978) and the dissolved oxygen content of the water monitored immediately after the lights turned on in the morning, immediately after the lights turned off in the evening, and immediately after the light turned on the following morning. This data was used to calculate production, respiration, and net daily metabolism within each beaker according to the method of McConnell (1962).

Statistical differences between treatment groups were evaluated using 2 way ANOVA. Significance of the interaction suggests that communities developed with and without stress are responding differently to the subsequent stress, e.g., adaptation or decreased resistance may be occurring. In absence of interaction, the significance of the main effect for pretreatment suggests that communities colonized under stress were different from those colonized with no added toxicant. Similarly, significance of the main effect for posttreatment suggests that that the second stress significantly affected communities.

In full scale comparative toxicity tests two types of effect levels were calculated to compare the relative response of pretreatment groups. LOECs were calculated separately for each pretreatment group using one-way ANOVA and Duncan's multiple range test. IC20 values were calculated by interpolating between adjacent values using a log scale.

Differences in taxonomic composition of communities were examined with Hendrickson's M statistic (Hendrickson 1978) which compares the number of positive matches in taxa for all pairs of substrates. Comparisons were made stepwise, by recalculating the statistic after sequential elimination of the highest treatment

group and a correlation in the number of positive matches in two or more of substrates was no longer significant. Control substrates from both pretreatment groups were also compared. Core taxa were defined as those species present on all three replicates of a treatment. Jaccard's coefficient was calculated for core taxa in each treatment group and clustered using an average linkage method (Pinkham and Pearson 1976).

Nominal concentrations of stress were confirmed analytically at intervals. Cadmium concentrations in the water column were determined by graphite furnace atomic absorption spectrophotometry. Zinc concentrations in the water column were determined by flame atomic absorption spectrophotometry. Nominal concentrations were used for these preliminary analyses.

## Results

In the full scale acute test, mean species richness was different in the two pretreatment groups ( $40.2 \pm 4.2$  vs  $33.8 \pm 4.6$ , mean  $\pm$  1 standard deviation for unstressed and cadmium stressed, respectively). The observed 16% inhibition in species richness after colonization in  $1 \text{ ug Cd L}^{-1}$  was close to the predicted value of 20%. But differences in richness were less pronounced at higher posttreatment levels (Figure 2) and the interaction between pretreatment and posttreatment was not significant ( $p = 0.1161$ , Table 3). Algal biomass as monitored by  $^{14}\text{C}$  was quite variable and showed significant declines only at the highest posttreatment levels, with no significant interaction (Table 3 and Figure 3). Taxonomic composition was not significantly different in communities from the two pretreatment

groups initially, but posttreatment exposures to zinc did have significant effects on composition (Table 3) and treatments clustered out according to posttreatment concentrations rather than pretreatment (Figure 4).

Results of the full scale chronic test are summarized in Table 4 and Figures 5-10. In contrast to the acute test, no significant differences between pretreatment groups were identified (Table 4). Species richness of control communities was  $41.3 \pm 4.9$  vs  $41.7 \pm 5.7$  for control vs cadmium stressed, respectively. Subsequent zinc exposure strongly affected species richness, algal biomass (IVF), morning dissolved oxygen, and taxonomic composition, but stressed and unstressed communities responded in similar manners, all decreasing with increasing zinc concentration in both pretreatment groups (Figures 5-7). Total biomass (AFDW, Figure 8) and respiration to biomass ratios (Figure 9) were too variable to permit meaningful conclusions, although there was a trend towards increased respiration to biomass ratios with increasing concentration, as expected. Comparisons of LOECs (Table 4) for pretreatment groups show no consistent trend. Species composition (Figure 10) was most strongly influenced by high zinc levels in posttreatment.

Because the early full scale tests did not identify significant differences in responses of stressed and unstressed groups, subsequent effort was focused on screening a wider variety of developmental stress levels and subsequent acute stresses.

Results of tests screening the responses of communities developed under 3 levels of zinc stress to a variety of secondary stresses are summarized in Table 5 and Figures 11-16. Most interaction terms were insignificant, suggesting no difference in response between stressed and unstressed groups. However, interaction terms were significant for 6 out of 36 responses monitored. The net daily metabolism of zinc stressed communities was less affected by subsequent zinc exposure, suggesting acclimation (Figure 11). Similarly, production and respiration were severely reduced after cadmium exposure but changes were less pronounced in zinc stressed communities than in the control (Figure 14). When exposed to acidic pH, species richness was less affected in communities previously exposed to zinc than in control communities (Figure 15), and, when this test was repeated, similar results were obtained (Figure 16). Total biomass (AFDW) after exposure to acidic pH was also less affected in previously stressed groups for the first test (Figure 15).

## Conclusions

Following is a summary of our progress in terms of the original objectives.

Objective 1: Do communities with a history of stress respond differently to subsequent stress than do unstressed communities?

To date we have seen no effect of pretreatment at minimal levels of cadmium stress on subsequent sensitivity to acute or chronic zinc exposure. We think that a more severe stress is needed to induce an acclimation at the community level that we are able to detect. This is consistent with the observations of

Weis and Weis (1989) and others who suggest that there is no acclimation at the population level until substantial damage has occurred, i.e., adaptation is not without costs. In our tests, communities exposed to any stress were generally less rich taxonomically, with lower rates of production and respiration, although they sometimes were indistinguishable in terms of standing crop.

Screening tests of the acute sensitivity of communities pretreated with higher levels of zinc stress show both functional acclimations (net daily metabolism, production, respiration) and structural acclimations (species richness, total biomass). And an acclimation in one response was not accompanied by similar acclimations in other responses.

Objective 2: Is adaptation generalizable from one stress to another?

Screening tests to date have shown cross adaptation for communities developed in zinc and exposed to cadmium and pH.

Objective 3: What are possible mechanisms of observed adaptations?

Possible mechanisms of adaptation will be examined in follow up tests.

Objective 4: What are commonalities between chemicals to which communities are observed to adapt?

Additional screening tests are necessary before this question can be addressed.

## FUTURE PLANS

We are focusing continued efforts on acute screening tests with communities developed under relatively severe stress followed by full scale tests for combinations of stress resulting in a significant adaptation or decrease in resistance. Possible mechanisms of response, e.g., congeneric homeotaxis, will be examined in full scale tests. The information from screening tests will be combined to evaluate patterns in occurrence of acclimation, as described in the original proposal. We are currently colonizing substrates for full scale acute tests with zinc followed by zinc, and zinc followed by acid. We are also analyzing patterns over time in communities developed in zinc over a long time period to differentiate early acclimation from responses taking longer to develop.



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Table 1: Criterion concentrations for chronic stress based on regressions of species richness (logit transformed) on concentration (log transformed).

Stress	IC20	IC30	IC50	Reference
Ammonia (Un-ionized) (ug L <sup>-1</sup> )	1.2	6.7	66.5	Cairns et al. 1988
Cadmium (ug L <sup>-1</sup> )	1.1	2.2	6.6	Niederlehner et al. 1985
Chlorine (ug L <sup>-1</sup> )	7.6	17.8	72.8	Pratt et al. 1988
Copper (ug L <sup>-1</sup> )	8.3	12.2	22.7	Pratt et al. 1987a
Diquat (mg L <sup>-1</sup> )	0.2	0.4	2.1	Pratt et al. 1989
pH (units)	6.65	6.01	5.00	Cairns et al. 1988
Zinc (ug L <sup>-1</sup> )	20.7	51.1	228.2	Pratt et al. 1987b

Table 2: Results of range-finding tests to find the degree of stress necessary to reduce the species richness of a substrate community by 50% within 48 h. Table entries are concentrations followed by protozoan species richness.

Stress	Control	Low	Medium	High	IC50
Ammonia-Total (mg L <sup>-1</sup> )	<0.02 36	10 27	100 10	1,000 2	35
Cadmium (ug L <sup>-1</sup> )	<2 33	200 16	1,000 4	6,000 2	138
Chlorine (ug L <sup>-1</sup> )	0 44	- -	10 27	100 1	13
Diquat (mg L <sup>-1</sup> )	0 36	1 15	3 11	10 5	0.7
p-Nitrophenol (mg L <sup>-1</sup> )	0 37	1 31	10 28	100 16	74
pH (units)	8.0 37	5.0 47	4.0 27	3.5 7	3.8
Sodium (mg L <sup>-1</sup> )	4 37	10 34	100 28	1,000 7	230
Temperature change (° C)	0 44	20 20	30 12	40 1	14
Zinc (ug L <sup>-1</sup> )	4 40	500 32	2,000 23	8,000 6	1,968

Table 3: Comparison of responses of unstressed (U) and cadmium stressed (S) communities to subsequent acute zinc exposure.

Response	LOECs	IC20s	p-values		
	U vs S	U vs S	pre-treat- ment	post-treat- ment	inter- action
Species Richness	$\leq 500$ vs $\leq 500$	$\leq 500$ vs $\leq 500$	0.0065	0.0001	0.1161
IVF	>16,000 vs 16,000	vs	0.2713	0.0112	0.9623
Taxonomic Composition	$\leq 500$ vs $\leq 500$	NC	0.1993	0.0001	NC

NC=not calculated

Table 4: Comparison of responses of unstressed (U) and cadmium stressed (S) communities to subsequent chronic zinc exposure.

Response	LOECs	IC20s	p-values		
	U vs S	U vs S	pre-treat- ment	post-treat- ment	inter- action
Species Richness	320 vs $\leq 10$	159 vs 100	0.1945	0.0001	0.6915
Ash Free Dry Weight	>3,200 vs >3,200	NC	0.3726	0.0813	0.8900
IVF	100 vs 3,200	49 vs 409	0.8800	0.0001	0.4207
Morning Dissolved Oxygen	320 vs 320	359 vs 421	0.6141	0.0001	0.9993
Respiration to Biomass Ratio	>3,200 vs >3,200	NC	0.8015	0.2840	0.8495
Taxonomic Composition	32 vs $\leq 10$	NC	0.6348	0.0001	NC

NC=not calculated

Table 5: ANOVA results comparing responses of communities developed under three levels of zinc stress to subsequent stress at an acute IC50. Table values are p-values. Abbreviations are as follows: AFDW=ash free dry weight, IVF=in vivo fluorescence, NDM=net daily metabolism, GPP=gross primary productivity, CR=community respiration.

Post-treatment: Response	Pre-treatment Main Effect	Post-treatment Main Effect	Interaction
Zinc:			
Richness	0.0310	0.0054	0.8168
AFDW	0.1001	0.5793	0.7570
IVF	0.2231	0.6139	0.3810
NDM	0.0042	0.0021	0.0230
GPP	0.7841	0.0142	0.3393
CR	0.1260	0.1032	0.9016
p-Nitrophenol:			
Richness	0.0011	0.0001	0.4945
AFDW	0.1161	0.9610	0.3479
IVF	0.4693	0.0001	0.5908
NDM	0.1797	0.0259	0.1749
GPP	0.3773	0.6349	0.8728
CR	0.3534	0.9874	0.9122
Sodium Chloride:			
Richness	0.0001	0.0029	0.1122
AFDW	0.0009	0.1560	0.1404
IVF	0.0016	0.3364	0.8091
NDM	0.2741	0.3699	0.5387
GPP	0.0007	0.3247	0.2296
CR	0.0002	0.0710	0.1119
Cadmium:			
Richness	0.0001	0.0001	0.1123
AFDW	0.0019	0.1745	0.1571
IVF	0.0039	0.8512	0.8692
NDM	0.0271	0.0028	0.2400
GPP	0.0007	0.0012	0.0162
CR	0.0001	0.0038	0.0380

Table 5: continued

Post- treatment: Response	Pre- treatment Main Effect	Post- treatment Main Effect	Interaction
pH:			
Richness	0.0002	0.6627	0.0091
AFEX	0.0236	0.9298	0.0049
IVF	0.0143	0.2289	0.9138
NEM	0.9171	0.0016	0.5474
GPP	0.0932	0.0011	0.7473
CR	0.0387	0.0447	0.8072
pH repeated:			
Richness	0.1530	0.0188	0.0236
AFEX	0.9665	0.2750	0.1174
IVF	0.7601	0.1866	0.1744
NEM	0.0735	0.0002	0.2969
GPP	0.1731	0.0315	0.8596
CR	0.0339	0.6262	0.6899



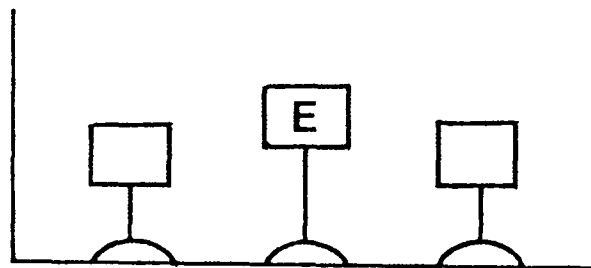
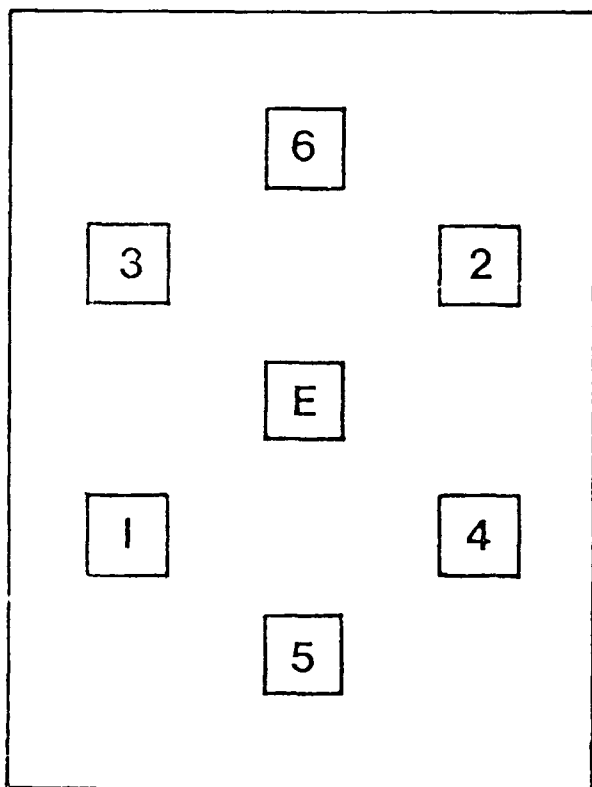


Figure 1: Test system diagram. A naturally-derived epicenter (E) is surrounded by six initially barren island substrates.

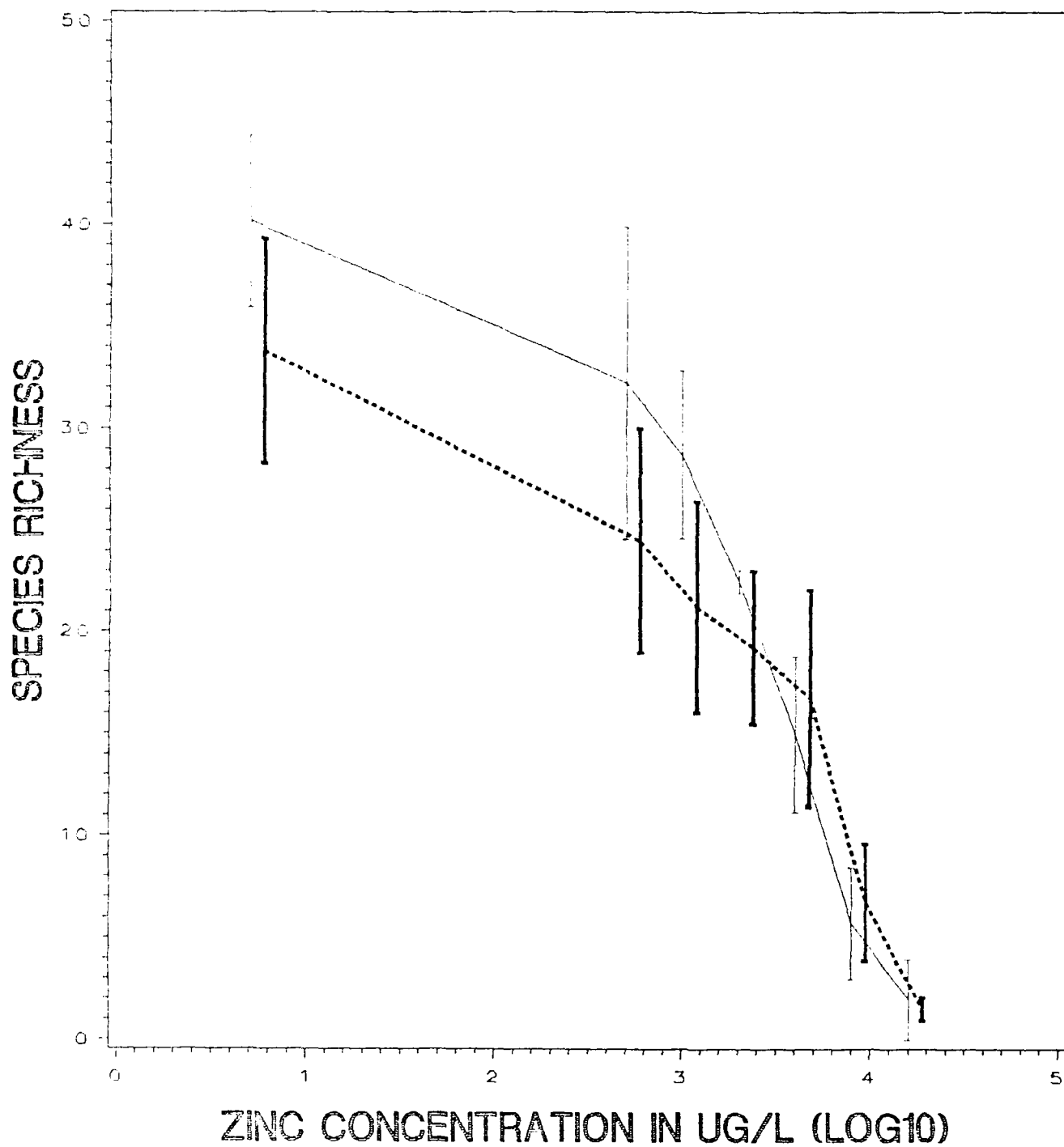


Figure 2: Effects of acute zinc exposure on the species richness of unstressed and cadmium stressed communities. Control communities are represented with a solid line; stressed communities with a dashed line. Bars represent one standard deviation.

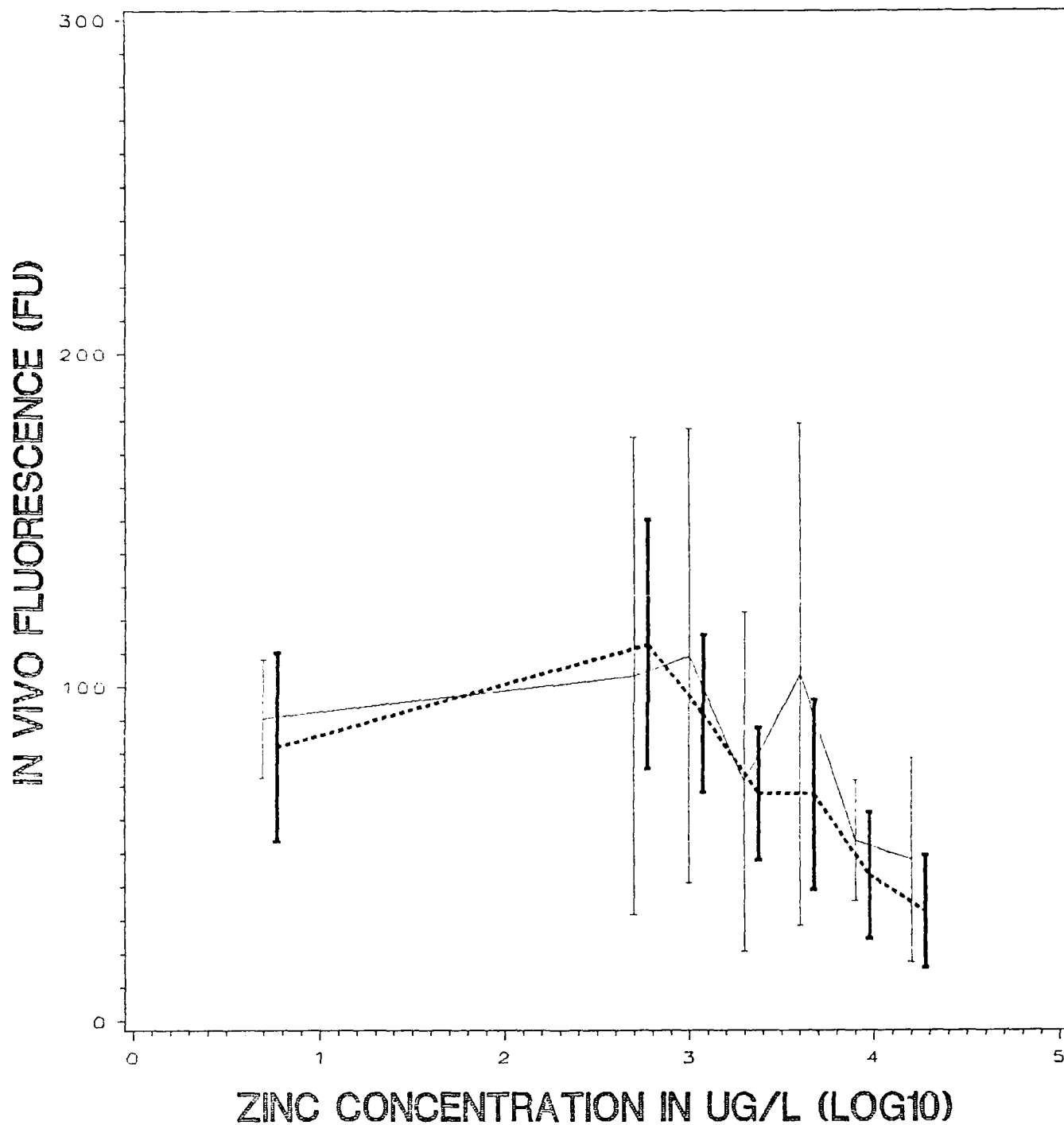


Figure 3: Effects of acute zinc exposure on the in vivo fluorescence of unstressed and cadmium stressed communities. Control communities are represented with a solid line; stressed communities with a dashed line. Bars represent one standard deviation.

DENDROGRAM PLOT NUMBER 1

CRANIUM THEN ZINC CHRONIC TEST CORE SPECIES

USING PINKHAM AND PEARSON COEFFICIENT OF ASSOCIATION,

0 0 MATCHES IGNORED

GROUP SIZE UNIMPORTANT

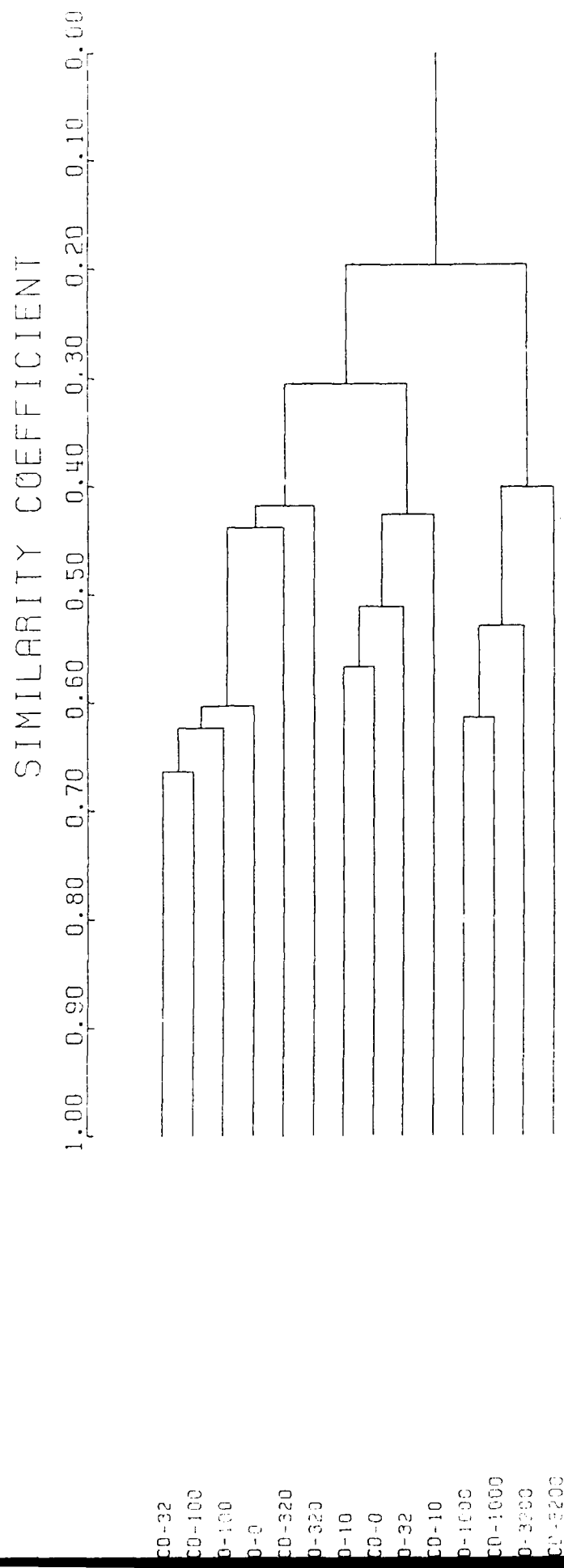


Figure 4: Dendrogram for acute zinc tests.

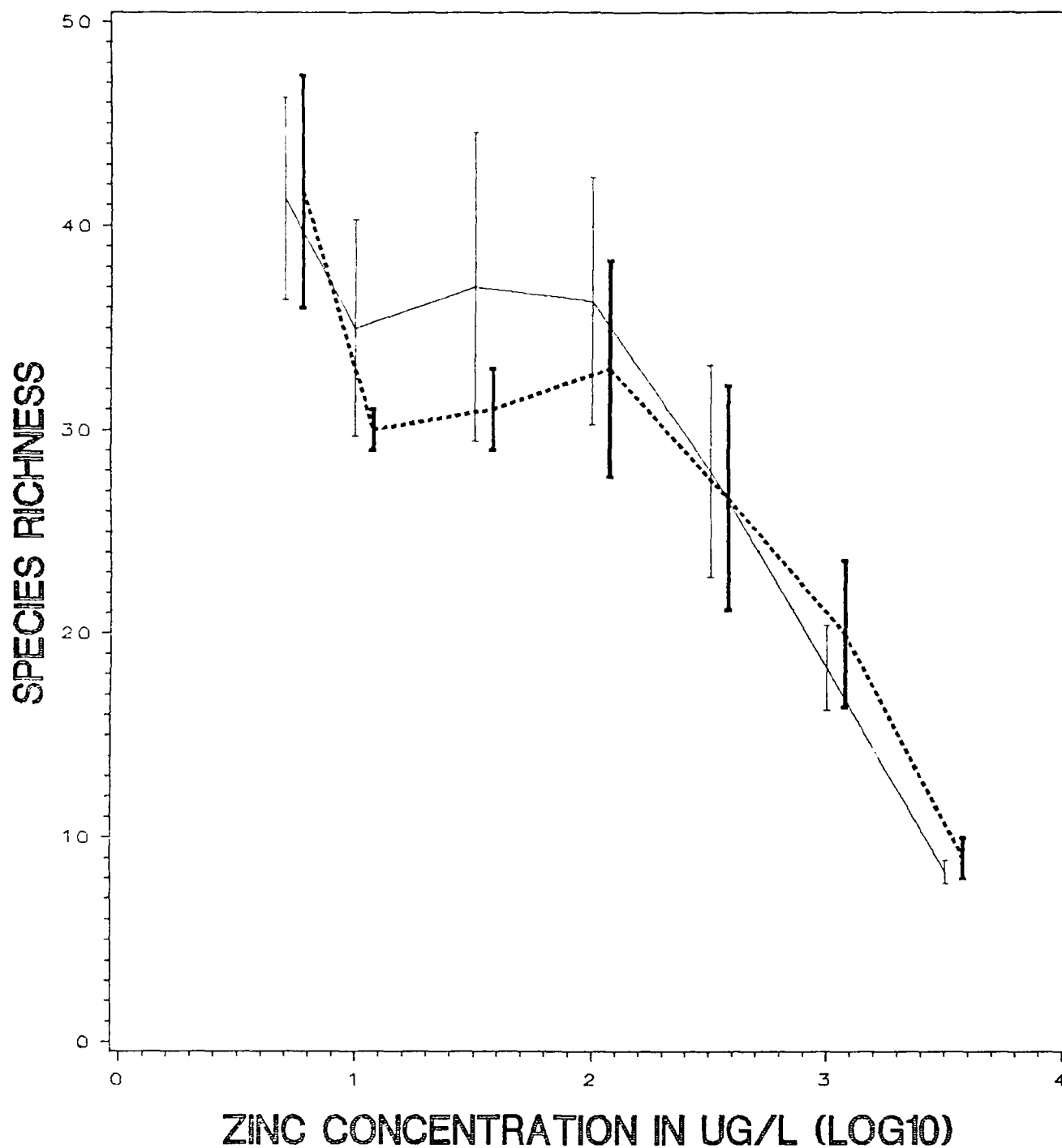


Figure 5: Effects of chronic zinc exposure on the species richness of unstressed and cadmium stressed communities. Control communities are represented with a solid line; stressed communities with a dashed line. Bars represent one standard deviation.

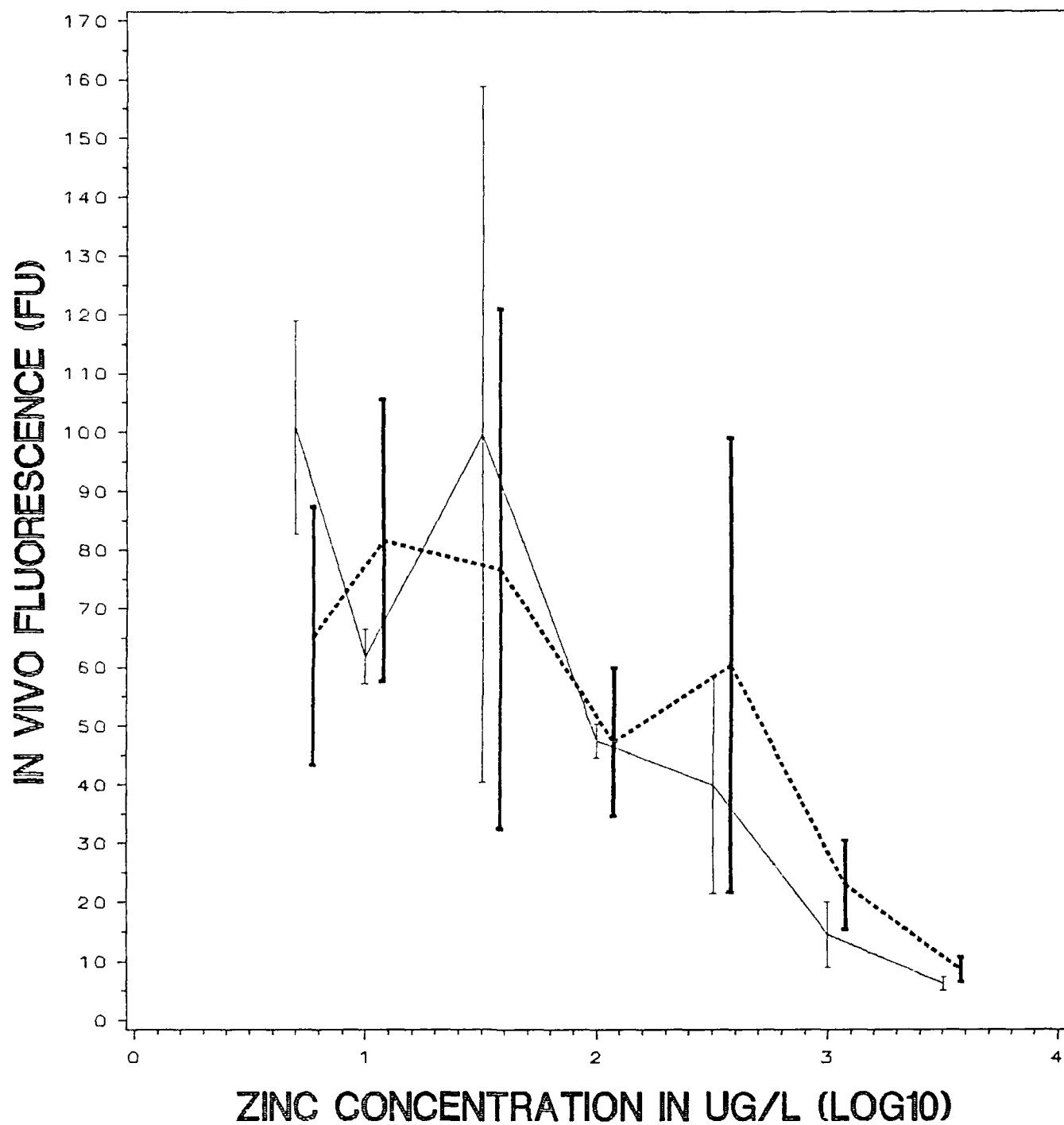


Figure 6: Effects of chronic zinc exposure on the *in vivo* fluorescence of unstressed and cadmium stressed communities. Control communities are represented with a solid line; stressed communities with a dashed line. Bars represent one standard deviation.

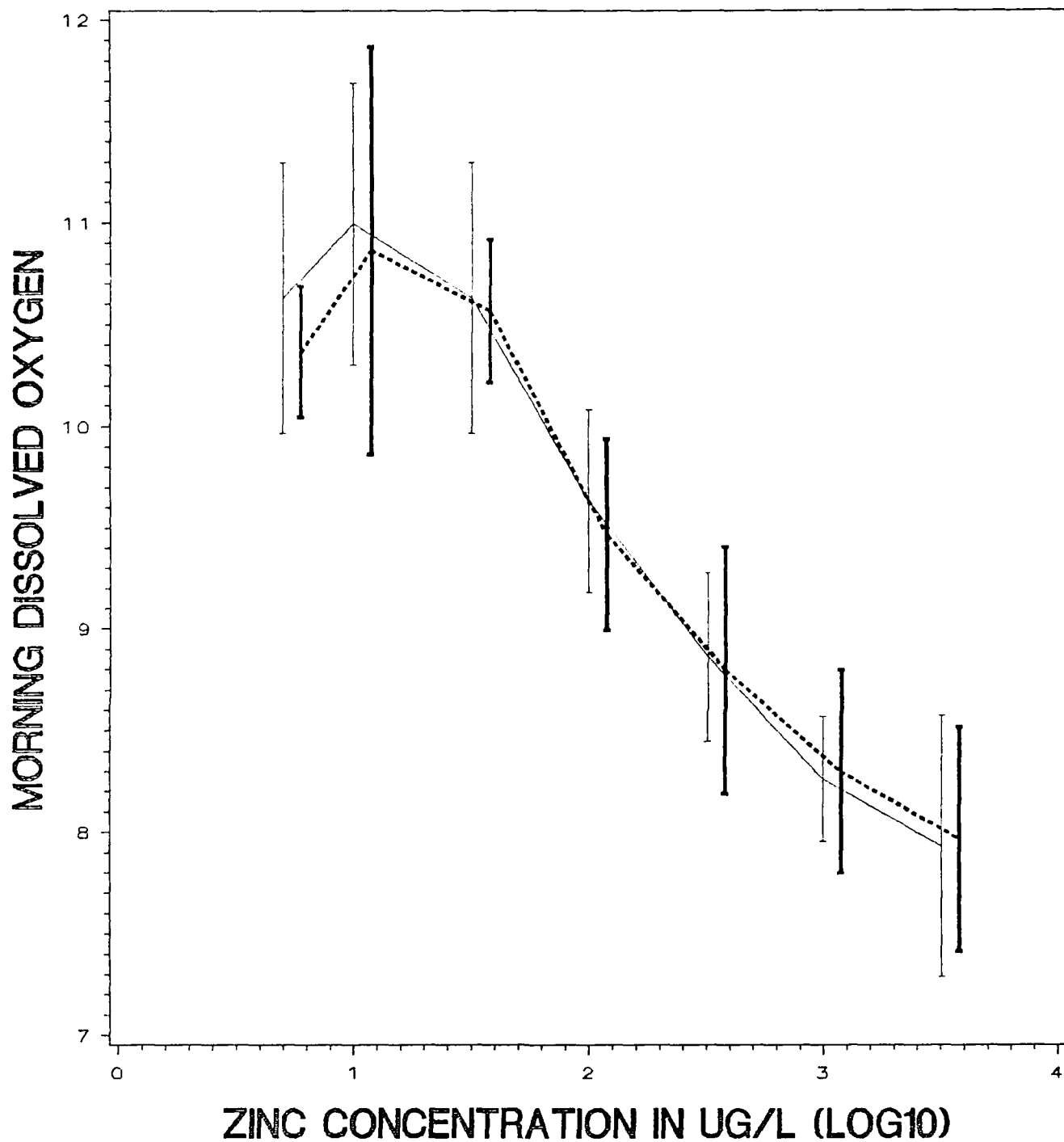


Figure 7: Effects of chronic zinc exposure on the morning dissolved oxygen content in  $\text{mg L}^{-1}$  of unstressed and cadmium stressed communities. Control communities are represented with a solid line; stressed communities with a dashed line. Bars represent one standard deviation.

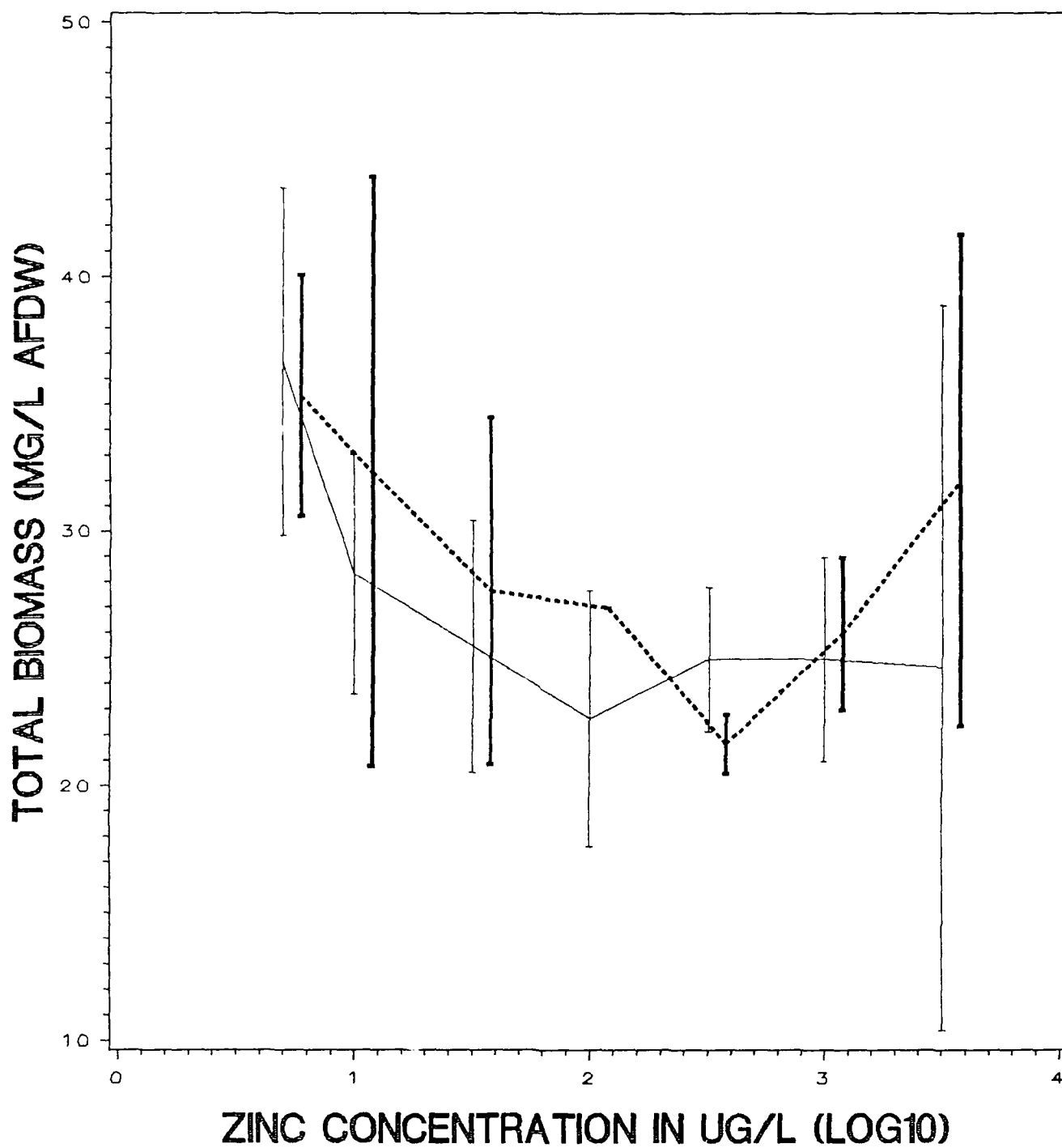


Figure 8: Effects of chronic zinc exposure on the ash free dry weight in  $\text{mg L}^{-1}$  of unstressed and cadmium stressed communities. Control communities are represented with a solid line; stressed communities with a dashed line. Bars represent one standard deviation.



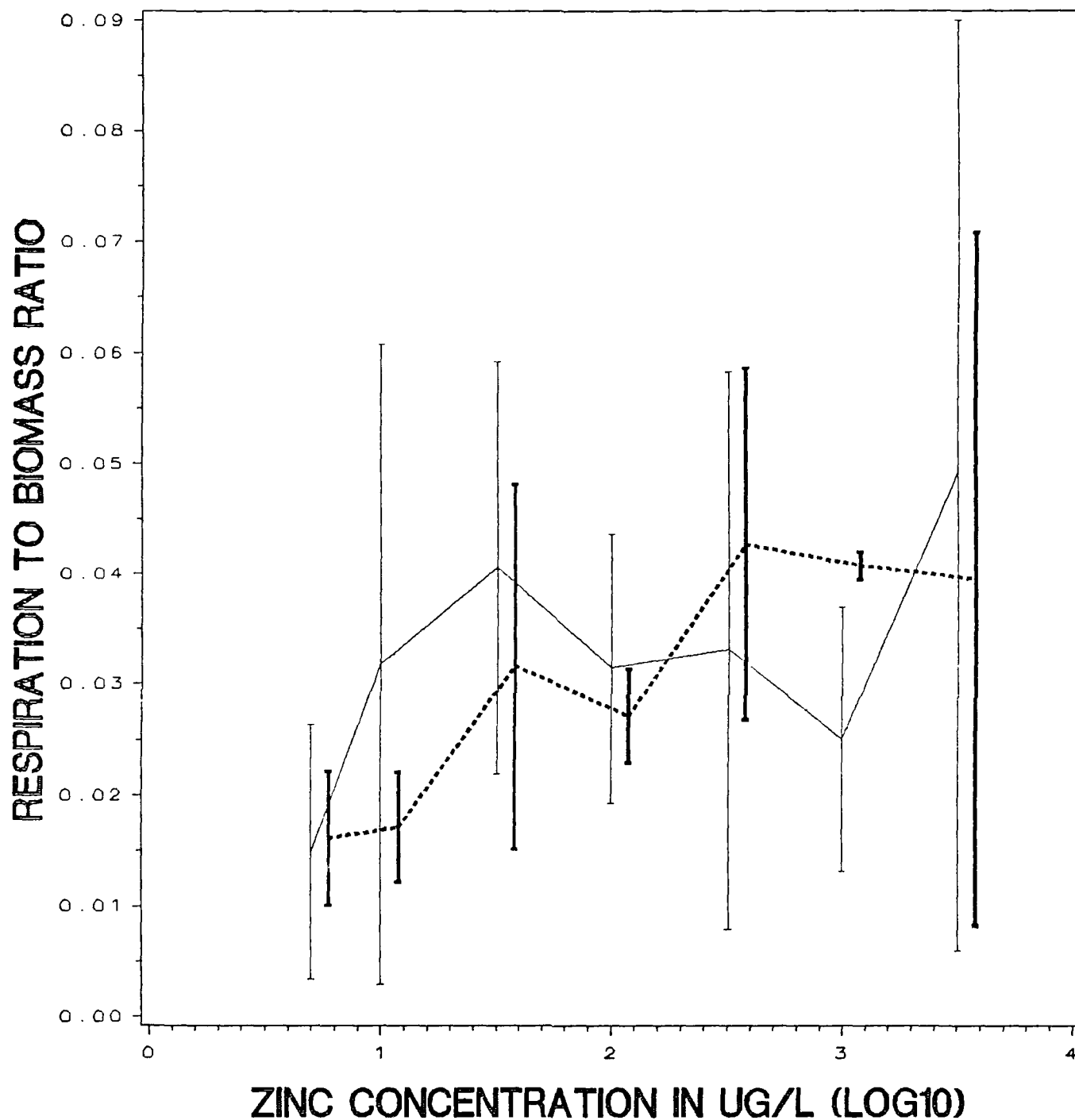


Figure 9: Effects of chronic zinc exposure on the respiration to biomass ratio in  $\mu\text{g O}_2 \text{ mg AFDW}^{-1} \text{ h}^{-1}$  of unstressed and cadmium stressed communities. Control communities are represented with a solid line; stressed communities with a dashed line. Bars represent one standard deviation.

DENDROGRAM PLOT NUMBER 1

CADMIUM ACCLIMATION THEN ZINC ACUTE CORE SPECIES C  
USING PINKHAM AND PEARSON COEFFICIENT OF ASSOCIATION,  
0 0 MATCHES IGNORED  
GROUP SIZE UNIMPORTANT

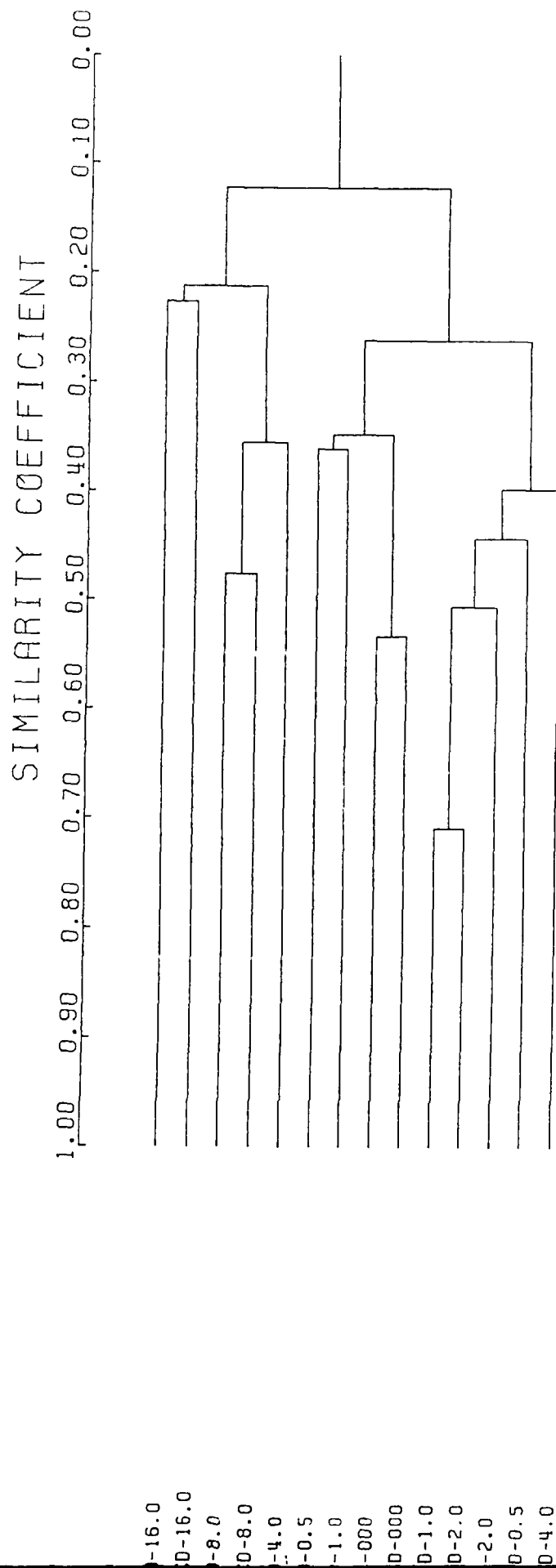


Figure 10: Dendrogram for chronic zinc test.

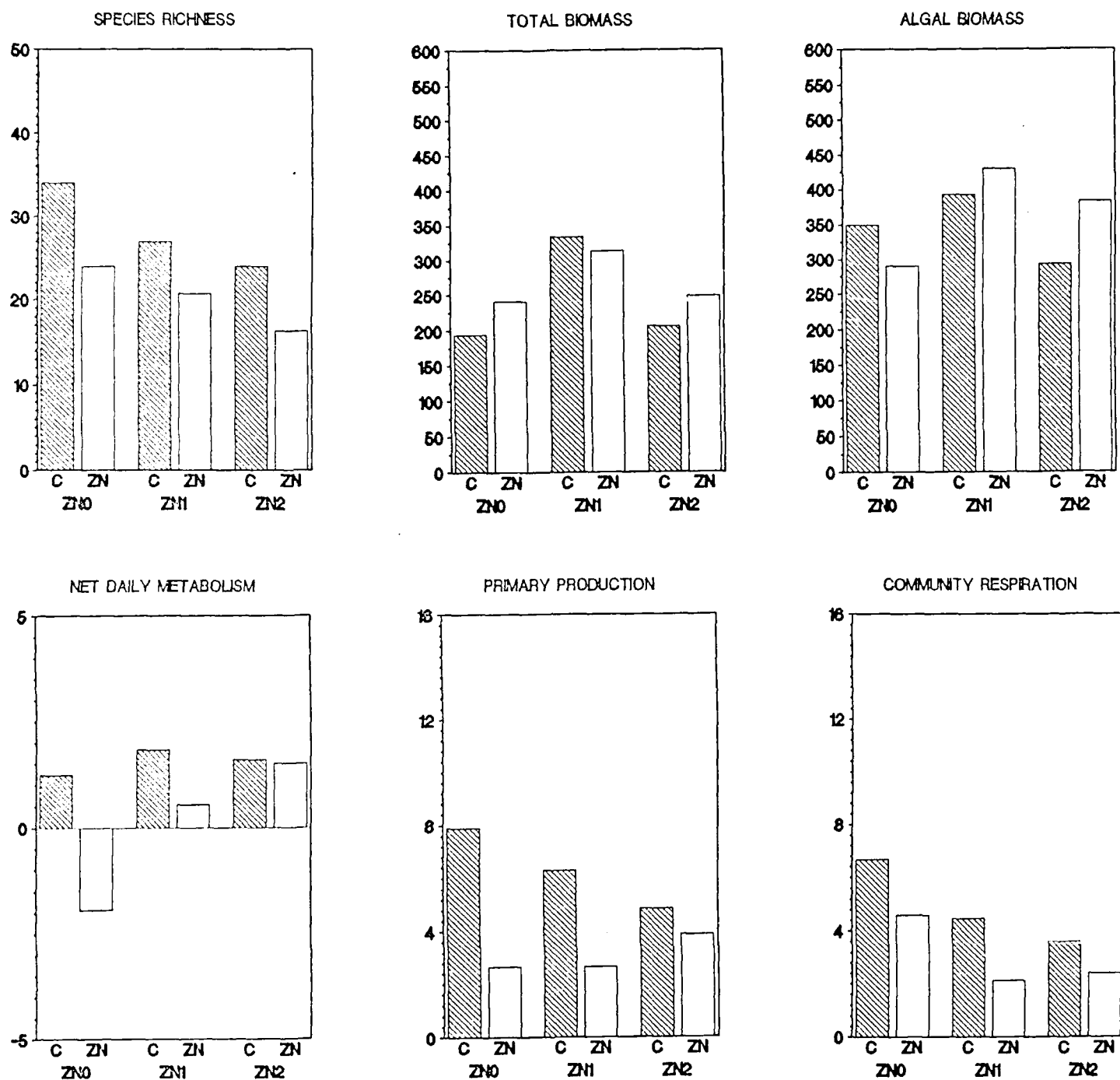


Figure 11: Effects of acute zinc exposure on responses of unstressed and zinc stressed communities. ZN0, ZN1, and ZN2 refer to the three pretreatment groups. The control posttreatment group (C) is represented by a shaded bar. The stressed posttreatment group (ZN) is represented by an empty bar.

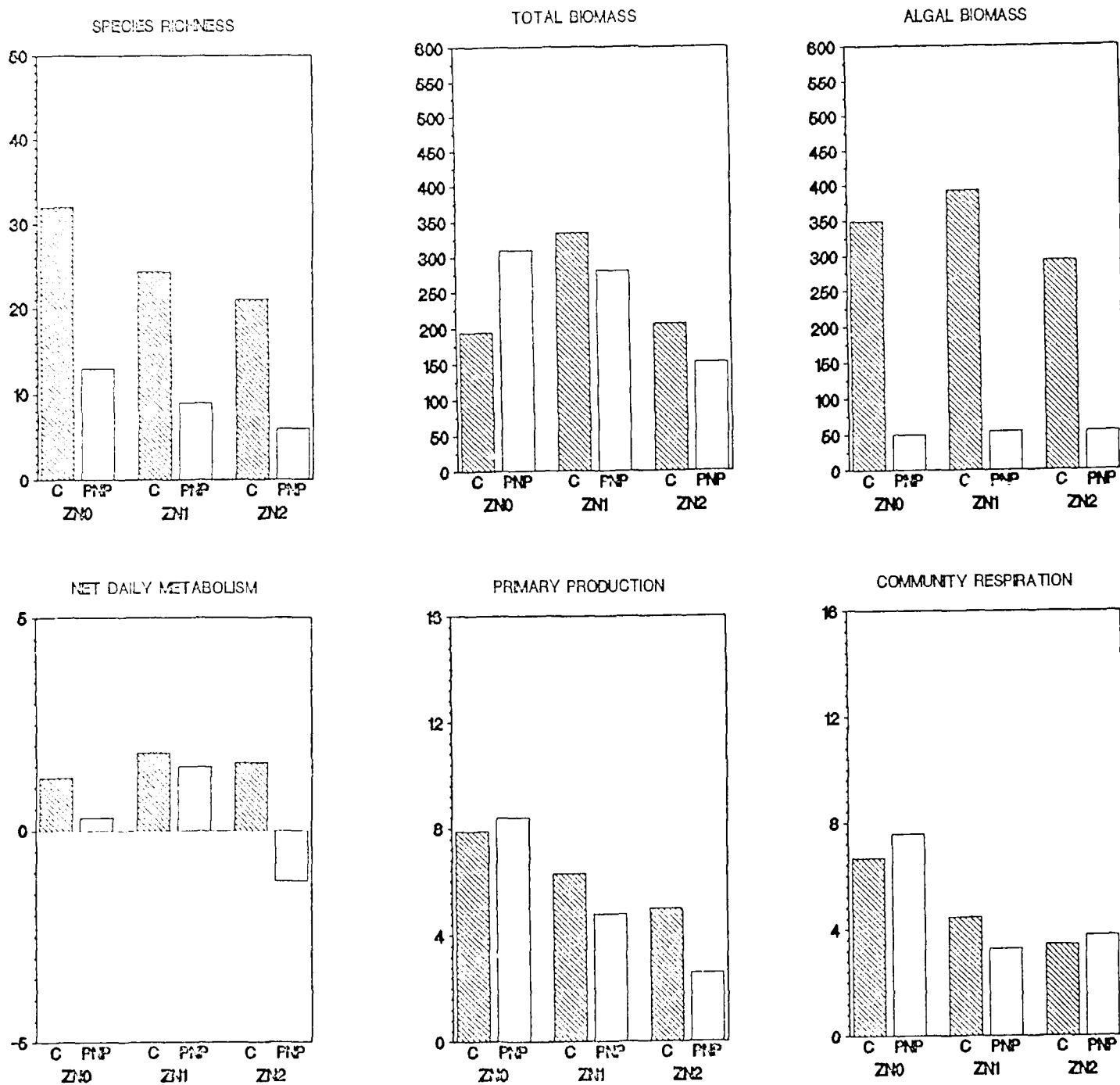


Figure 12: Effects of acute p-nitrophenol exposure on responses of unstressed and zinc stressed communities. ZN0, ZN1, and ZN2 refer to the three pretreatment groups. The control posttreatment group (C) is represented by a shaded bar. The stressed posttreatment group (PNP) is represented by an empty bar.

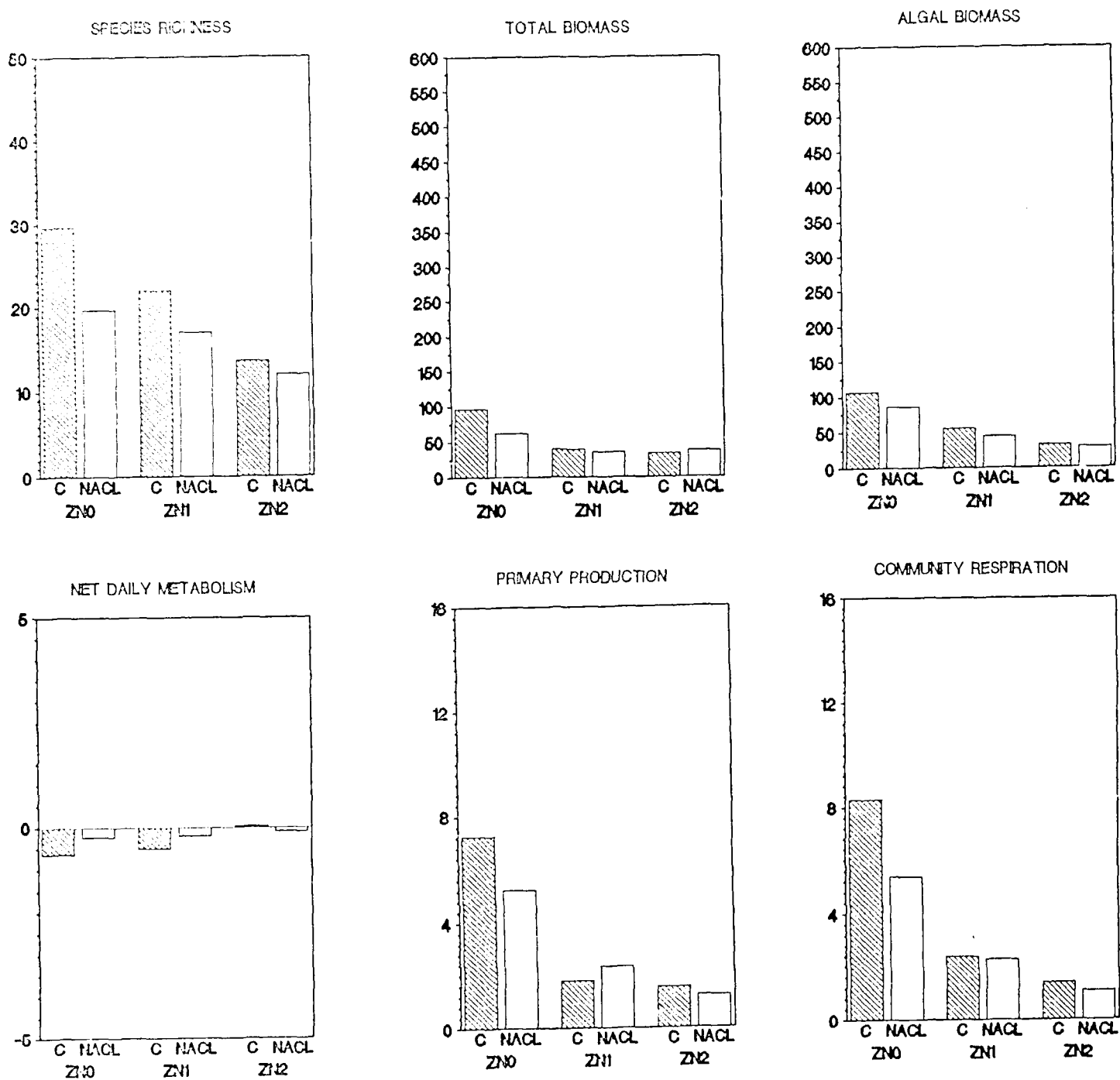


Figure 13: Effects of acute sodium chloride exposure on responses of unstressed and zinc stressed communities. ZN0, ZN1, and ZN2 refer to the three pretreatment groups. The control posttreatment group (C) is represented by a shaded bar. The stressed posttreatment group (NACL) is represented by an empty bar.

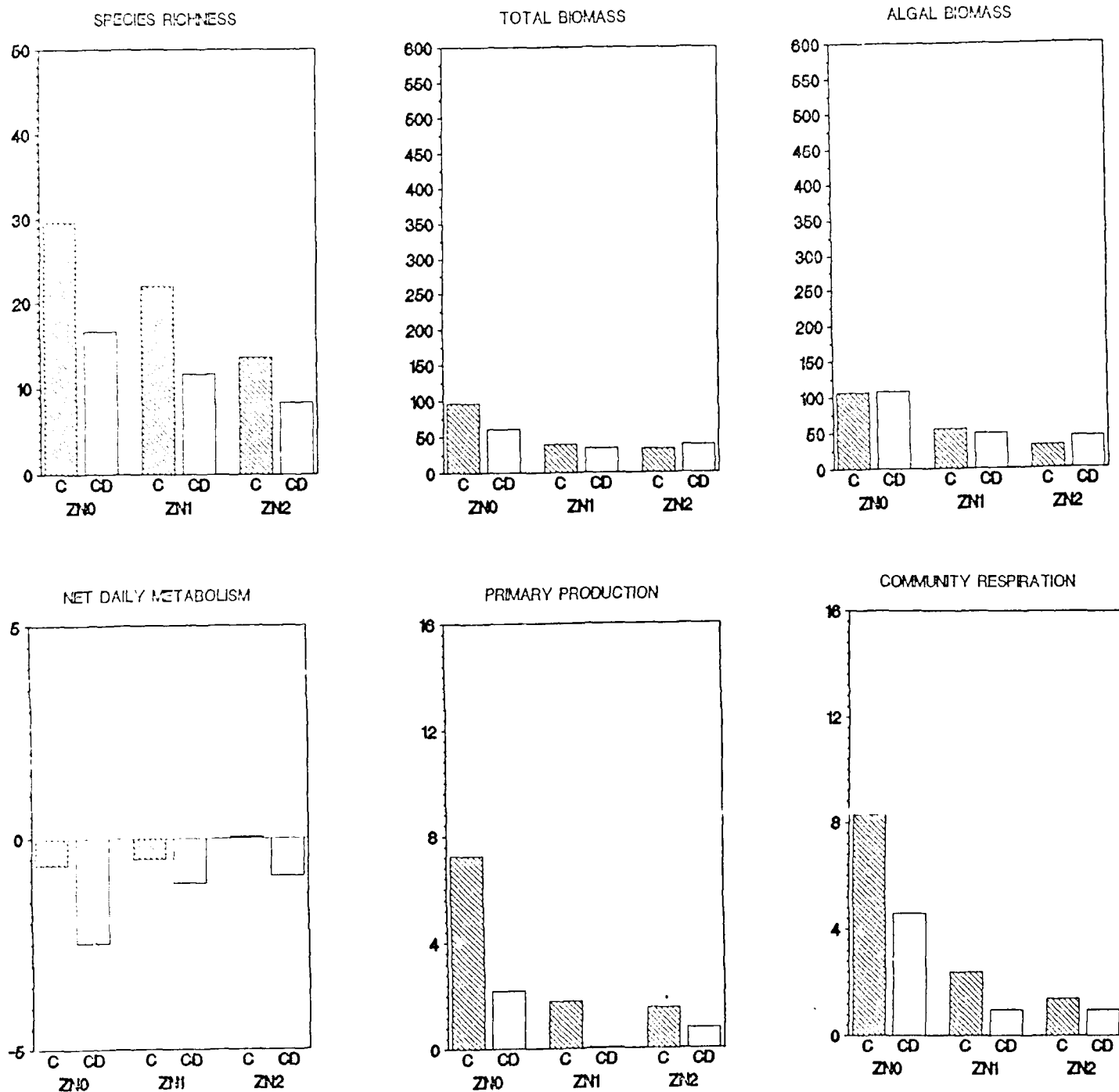


Figure 14: Effects of acute cadmium exposure on responses of unstressed and zinc stressed communities. ZN0, ZN1, and ZN2 refer to the three pretreatment groups. The control posttreatment group (C) is represented by a shaded bar. The stressed posttreatment group (CD) is represented by an empty bar.

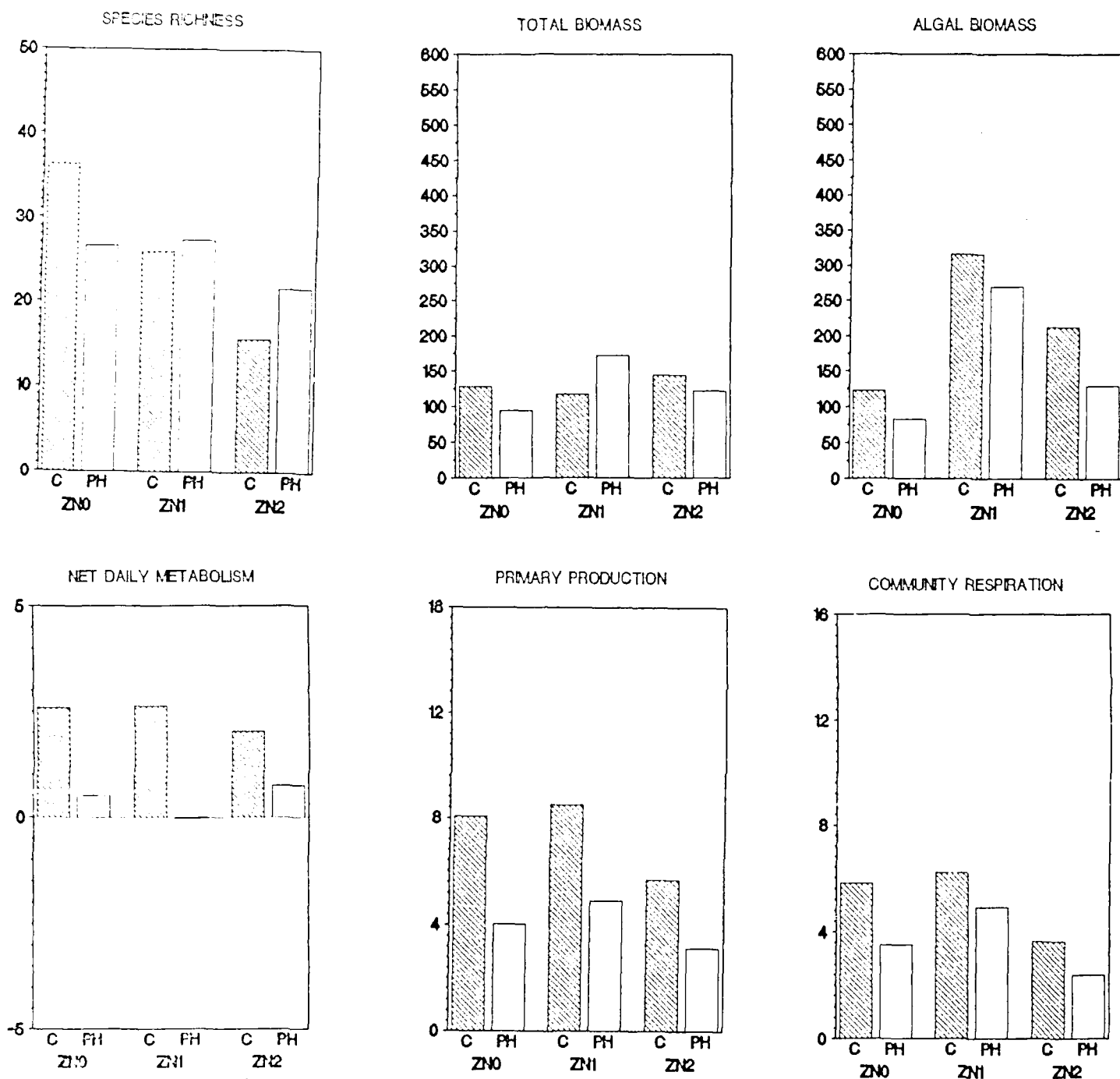


Figure 15: Effects of acute exposure to acidic pH on responses of unstressed and zinc stressed communities. ZN0, ZN1, and ZN2 refer to the three pretreatment groups. The control posttreatment group (C) is represented by a shaded bar. The stressed posttreatment group (PH) is represented by an empty bar.

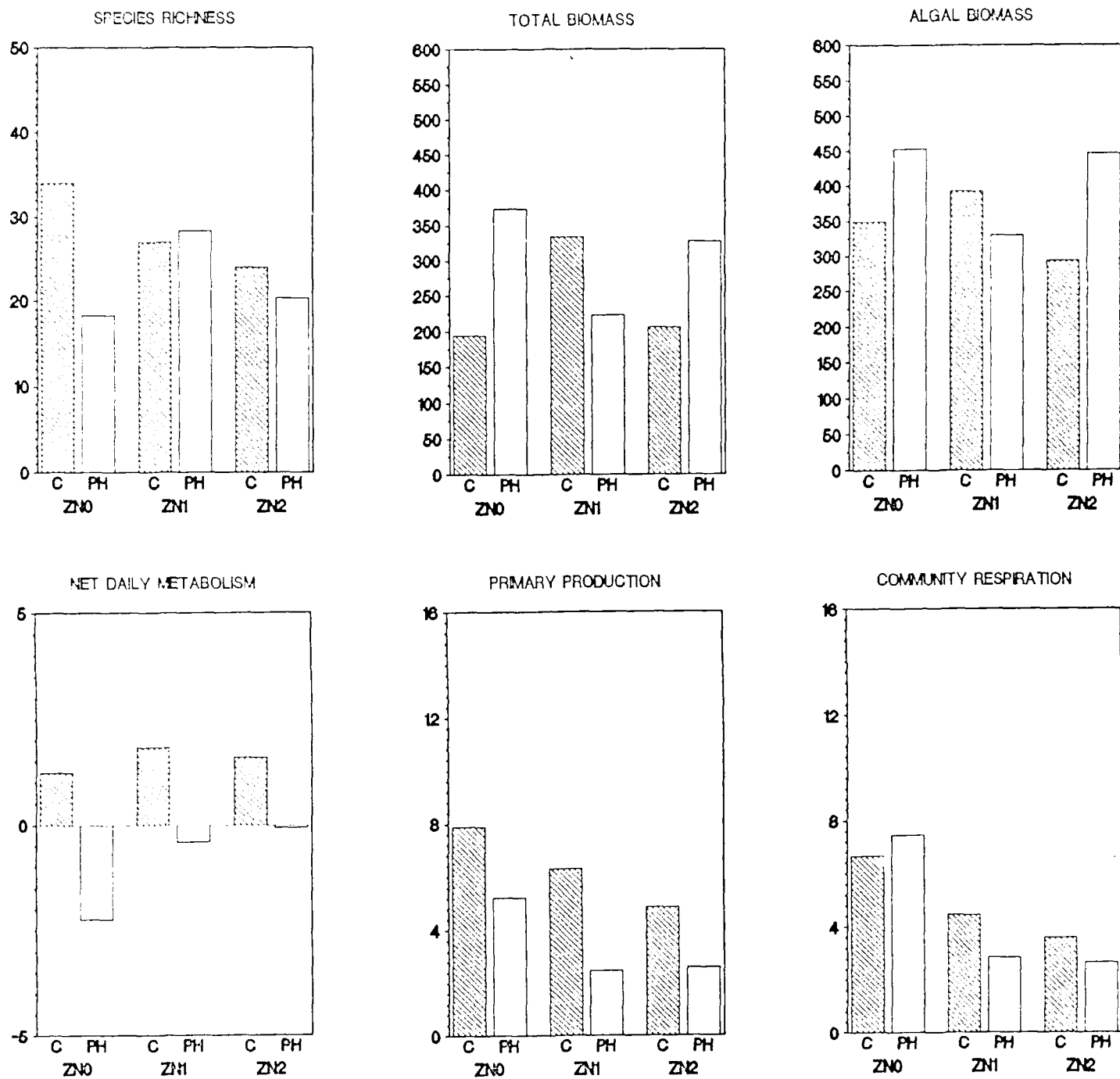


Figure 16: Effects of acute exposure to acidic pH on responses of unstressed and zinc stressed communities, repeated. ZN0, ZN1, and ZN2 refer to the three pretreatment groups. The control posttreatment group (C) is represented by a shaded bar. The stressed posttreatment group (PH) is represented by an empty bar.



#### PERSONNEL

Dr. John Cairns, Jr., University Distinguished Professor and Director of the University Center for Environmental and Hazardous Materials Studies, serves as principal investigator for this project.

Ms. Barbara R. Niederlehner, laboratory specialist, serves as co-investigator. Half of her time is devoted to this project and half of her salary is paid for by this project.

An undergraduate student in biology, Ms. Jeaninne Engle, has assisted in data collection and analysis.

#### PUBLICATIONS

Cairns, John, Jr., and B. R. Niederlehner. In preparation.  
Effects of zinc acclimation on response of periphytic communities  
to subsequent stress. Abstract prepared for North American  
Benthological Society Annual Meeting. May 1990, Blacksburg, VA.